Indolebutyric acid and ectomycorrhizal inoculation increase lateral root initiation and development of container-grown black oak seedlings

C. M. Baser, H. E. Garrett, R. J. Mitchell, and G. S. Cox
School of Forestry, Fisheries and Wildlife, University of Missouri, Columbia, MO, U.S.A. 65211

AND

C. J. Starbuck
Department of Horticulture, University of Missouri, Columbia, MO, U.S.A. 65211

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Containerized black oak seedlings in which the growth medium was inoculated with *Pisolithus tinctorius* (Pers.) Coker Couch or left uninoculated were grown for 16 weeks with or without a polyacrylic starch containing 1000 μg indolebutyric acid per gram mixed throughout the growth medium. Seedlings treated with indolebutyric acid developed more than twice as many lateral roots as control seedlings which did not receive indolebutyric acid. Indolebutyric acid reduced the percentage of primary laterals colonized by *P. tinctorius*; however, the total number of colonized lateral roots was not affected. The combination of ectomycorrhizal inoculation with the indolebutyric acid treatment significantly increased the growth and development of black oak seedlings.

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Les auteurs ont cultivé en récipients pendant 16 semaines, des plants de chênes noirs qu'ils ont inoculés ou non avec le *Pisolithus tinctorius* (Pers.) Coker et Couch et dans le substrat desquels ils ont mélangé 1000 μg d'acide indolebutyrique par gramme enrobé dans un amidon polyacrylique. Les plants traités avec l'acide indolebutyrique ont formé plus de deux fois plus de racines latérales que les plants témoins n'ayant pas reçu d'acide indolebutyrique. L'acide indolebutyrique a aussi réduit le pourcentage de racines primaires latérales transformées en mycorhizes par le *P. tinctorius*; cependant le nombre total de racines latérales colonisées n'a pas été affecté. La combinaison de l'inoculation ectomycorrhizienne avec le traitement à l'acide indolebutyrique a augmenté significativement la croissance et le développement des plants de chêne noir.

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Introduction

Natural regeneration of oak stands is dependent on the number and distribution of seedlings comprising the advanced reproduction. If advanced oak reproduction is inadequate, artificial regeneration must be employed to maintain the oak component in oak stands; however, artificial regeneration by direct seeding or planting bare-rooted stock often is unsuccessful owing to rodent damage and poor survival and growth of seedlings. In contrast, container-grown oak seedlings survive better and grow more rapidly than bare-rooted seedlings, since they have superior lateral root development (Johnson 1974), and a more vigorous root system after outplanting (Dixon *et al.* 1981). Early growth of container-grown stock after outplanting could be further improved by increasing lateral root development. One method of achieving this is by treating the root systems with exogenous auxin (Fowells 1943; Maki and Marshall 1945; Carlson 1974; Moser 1978; Hartwig and Larson 1980; Selby and Seaby 1982).

Auxins have been applied to bare-rooted seedlings by a variety of methods. Direct application methods used with transplants such as dusting roots with an auxin–talc mixture, spraying roots with auxin solutions, or inserting auxin-impregnated toothpicks into roots (Moser 1978), however, are not applicable to container-grown seedlings because the root systems are not exposed. In previous unpublished experiments in our laboratory, immersing the root system of container-grown oak seedlings in auxin solutions resulted in inconsistent increases in lateral root initiation. Furthermore, unacceptably high levels of mortality resulted (Crownover 1983). Apparently the timing of the auxin treatment in relation to the seedling's physiological development is critical for obtaining a favorable response (Selby and Seaby 1982); however, the conditions necessary for optimization of a rooting response to auxins have not been adequately defined.

In addition to plant growth regulators, ectomycorrhizal inoculation of root systems has been shown to increase lateral root effectiveness by increasing seedling nutrient content (Mitchell *et al.* 1984) and drought resistance (Dixon *et al.* 1980), while improving growth and survival after outplanting (Dixon *et al.* 1981). Although application of plant growth regulators has been shown to increase endomycorrhizal infection (Azcon *et al.* 1978), little is known about the effects of auxins on ectomycorrhizae. Exogenous auxins have been shown to increase the flux of carbohydrates to the point of application (Patric and Wareing 1972). Since ectomycorrhizal fungi are dependent on the host's root system for carbohydrates, treatments that increase carbohydrate transport to the root system may enhance ectomycorrhizal development.

Owing to the importance of a vigorous, well-developed root system in determining the outplanting success of oak seedlings (Johnson 1984), methods that enhance root development should further increase the quality of container-grown oak seedlings. The purpose of this study was to determine the effects of indolebutyric acid (IBA), applied via a polyacrylic starch

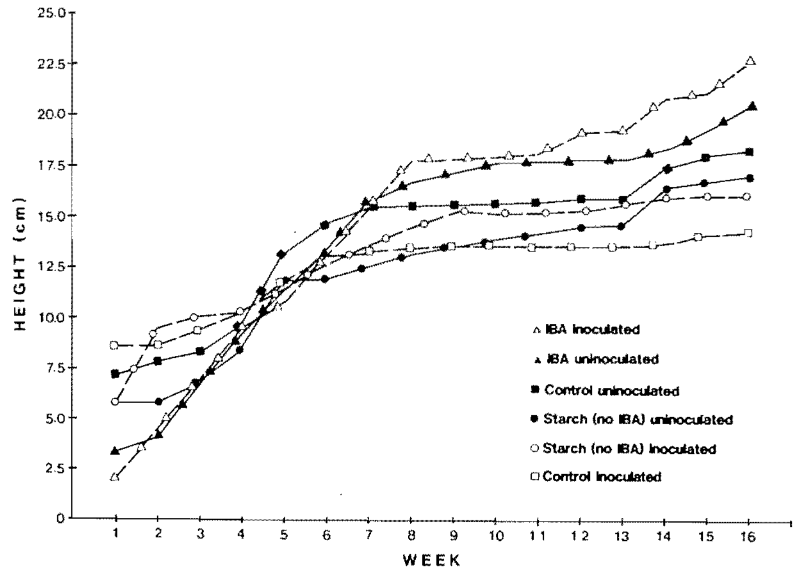

FIG. 1. Height of inoculated and uninoculated black oak seedlings in the presence or absence of IBA and polyacrylic starch.

carrier, on container-grown black oak seedlings; the effects of IBA upon ectomycorrhizal colonization and seedling development were also investigated.

Materials and methods

The study was conducted in a glasshouse at the University of Missouri – Columbia. Seedlings were grown for 16 weeks under artificial lighting (16-h photoperiod, 750 $\mu E \cdot m^{-2} \cdot s^{-1}$) at an average temperature of 25°C.

Black oak (*Quercus velutina* Lam.) acorns were collected from a single tree in late October in Columbia, Missouri. After soaking in water for 24 h, acorns which floated or had obvious weevil damage were discarded. Those remaining were stratified in plastic bags at 4°C for 65 days. After stratification, acorns were surface sterilized with a 10% Clorox bleach solution and allowed to germinate at room temperature.

A solution containing 1000 μg of IBA per gram was prepared by dissolving 1 g of IBA in 50 mL of ethanol and adding distilled water to volume (1 L). Ten grams of a polyacrylic starch (SGP 104K, Henkel Corp., Minneapolis, MN) was thoroughly mixed into the IBA solution to form an IBA–starch gel. A second starch compound was similarly produced without IBA. Ten litres of each gel was dried in an oven at 35–40°C for 50 days. Although some oxidation of the IBA may have occurred during the drying process, McDougall and Hillman (1978) report that solutions of indole compounds, such as IBA, can be dried under similar temperatures with minimal loss. After drying, the non-IBA starch and the IBA-containing starch were ground (20 mesh) with a Wiley mill and stored until used. Ectomycorrhizal inoculum of *Pisolithus tinctorius* (Pers.) Coker Couch, isolate 270 (secured from Don Marx, United States Department of Agriculture, Forest Service, Mycorrhizal Institute, Athens, GA), was grown for 10 months according to the methods described by Marx and Bryan (1975). The growth medium, which was sterilized with methyl bromide, consisted of a 1:1 (v/v) mixture of peat moss and construction-grade vermiculite. Thirty millilitres of either viable or autoclaved (120°C and 15 psi for 30 min) inoculum was thoroughly mixed with approximately 700 cm^3 of growth medium and placed in each of the three compartments (750 cm^3) comprising a Spencer-LeMaire Super 45 bookplanter. One pregerminated acorn was planted in each compartment.

Treatments consisted of adding (*i*) 2 g of the ground IBA-treated starch, (*ii*) 2 g of starch with no IBA, or (*iii*) no starch (control) to the growth medium of the containers inoculated with 30 mL of either viable or autoclaved *Pisolithus tinctorius* inoculum. The study was established in a complete randomized design with five replications per treatment. Each treatment replication consisted of the three seedlings in one bookplanter.

Seedlings received deionized water once a day for the first 7 weeks; from week 7 to the end of the experiment, water was applied every other day. Beginning the 4th week after planting, all seedlings were fertilized with 40 mL of one-half strength modified Hoagland's solution once each week (Bonner and Galston 1952).

Height measurements were recorded weekly following emergence. After a 16-week growing period, all seedlings were harvested and height, diameter, leaf area, stem dry weight, leaf dry weight, lateral root dry weight, and taproot dry weight were recorded. Additional root measurements included the number of taproots, primary lateral roots, ectomycorrhizal infected primary lateral roots, and ectomycorrhizal infection points per primary lateral root. Mycorrhizal colonization was determined by the methods described by Daughtridge *et al.* (1986). A staining solution was prepared by dissolving 0.15 g of Ponceau S (acid red 112) in 1 L of 90% distilled water and 10% glacial acetic acid. Root systems were stirred for 30 min in a 2-L jar containing the staining solution. Root systems were then rinsed in 10% acetic acid followed by a distilled water rinse. Each root system was cut in half perpendicular to the long axis of the taproot. The top or bottom half of each root system was randomly selected, primary lateral roots within that section were excised, and all infection points (highlighted by Ponceau S uptake) were counted under a dissecting microscope.

Data were analyzed by analysis of variance ($\alpha = 0.05$) and multiple comparisons were made by Duncan's multiple comparison test.

Results

IBA inhibited the early growth of container-grown black oak seedlings (Fig. 1). At the end of 1 week, control seedlings averaged 7.8 cm in height (7.1 for uninoculated, and 8.6 for inoculated seedlings). Seedlings in the IBA treatment, however, averaged only 2.7 cm (3.3 for uninoculated and 2.2 for inoculated). The IBA treatment also altered root morphology as early as week 1. IBA-treated seedlings were characterized by an increased number of taproots and the appearance of many small lateral initials. Seedlings in the control and starch (no IBA) treatments had single rapidly elongating taproots and few lateral initials. The early reduction in height growth of IBA-treated seedlings was followed by a period of rapid growth, so that seedling height within the IBA treatment exceeded that of the

TABLE 1. Aboveground measurements of 16-week-old mycorrhizal and nonmycorrhizal black oak seedlings receiving starch and IBA treatments

Treatment	Height (cm)	Diameter (mm)	Leaf area (cm^2)	Dry weight (g)	
				Stem	Leaf
Inoculated					
Control	14.0*d*	4.5*b*	301*c*	0.66*c*	2.29*c*
Starch	15.9*cd*	4.5*b*	323*c*	0.75*bc*	2.50*c*
Starch–IBA	24.0*a*	4.5*b*	480*a*	1.00*a*	3.80*a*
Uninoculated					
Control	18.5*bc*	5.1*a*	407*b*	0.98*a*	3.06*b*
Starch	17.5*cd*	4.8*ab*	404*b*	0.90*ab*	3.27*b*
Starch–IBA	21.2*b*	4.5*b*	403*b*	0.85*ab*	3.04*b*

NOTE: Means followed by the same letter are not significantly different at $\alpha = 0.05$.

control seedlings by week 7. After 16 weeks, the IBA-treated seedlings were 39% taller than control seedlings.

Mycorrhizae in combination with IBA significantly affected aboveground growth (Table 1). Height, stem dry weight, foliage dry weight, and leaf area were all increased, while stem diameter was unaffected. Although treatment with IBA generally stimulated growth of inoculated seedlings, its influence on uninoculated seedlings was minor.

IBA significantly affected total root dry weight, taproot dry weight, lateral root dry weight, and the number of primary lateral roots (Table 2). Total root dry weight and taproot dry weight were decreased by IBA, while the number of lateral roots was increased for both inoculated and uninoculated plants. Mycorrhizal colonization was significantly reduced by IBA (Table 2). The number of primary laterals colonized by *Pisolithus tinctorius* was not affected, but the percentage of total primary lateral roots colonized was significantly reduced. This reduction resulted from the greater number of laterals present on the IBA-treated seedlings. The number of infection points observed per primary lateral, however, was also reduced by IBA, and the morphology of the mycorrhizae was altered. The fungal mantle observed on the IBA-treated mycorrhizal short roots was diffuse and not apparent to the naked eye until stained. Well-developed mantles, which were readily visible, were characteristic of seedlings in the control and starch (no IBA) treatments. *Pisolithus tinctorius* fruiting bodies, however, were present on a few individuals in each of the treatments, including the IBA, indicating that the fungus was at least moderately vigorous under IBA-treated conditions.

Discussion

A decrease in the primary lateral root infection percentage was found in the IBA treatment in our study. However, in spite of the reduction observed, an effective symbiosis was apparent. While the percentage of lateral roots colonized was reduced by the IBA treatment, the number of primary laterals infected was slightly increased (80 for the IBA treatment, 75 for the control). Applying IBA resulted in approximately a 2.5-fold increase in black oak lateral root formation above control uninoculated seedlings not receiving IBA and almost a 3-fold increase in the number of lateral roots for non-IBA inoculated oak seedlings. Furthermore, significantly increased growth of the oak seedlings was observed in the IBA treatment.

The presence of ectomycorrhizal symbionts is an important factor in the outplanting success of oak seedlings (Kormanik 1976; Garrett *et al.* 1979); however, little is known about the effects of exogenous auxin applications on ectomycorrhizal colonization. Harley and Smith (1984) suggested that indole-3-acetic acid may inhibit ectomycorrhizal colonization of roots. Crownover (1983) reported decreased mycorrhizal colonization of oak seedlings soaked in IBA solutions.

Growth response of seedlings receiving ectomycorrhizal fungal inoculation and IBA treatments suggest a possible alteration in carbon partitioning within the seedling. Early height growth of IBA-treated seedlings was reduced, but after 16 weeks of growth, IBA-treated seedlings were significantly taller than control seedlings. Furthermore, IBA treatment in association with ectomycorrhizal inoculation provided greater stimulation in growth than either IBA or mycorrhizae alone.

The root:shoot ratio of IBA-treated seedlings was lower than that observed for control seedlings. Uninoculated control seedlings had a root:shoot ratio of 0.99, while uninoculated IBA-treated seedlings yielded a ratio of 0.83. The difference in root:shoot ratio was further altered by IBA application to inoculated seedlings (1.38 for control seedlings, 0.56 for IBA treated seedlings). The increase in the proportion of aboveground biomass in seedlings receiving IBA may be a result of IBA increasing the proportion of lateral root to taproot mass. Oak taproots, although large in weight, do not contribute as much to the uptake of water and nutrients as lateral roots. Van Sambeek and Rietveld (1983) also reported an increase in proportion of lateral root weight to taproot weight following

TABLE 2. Root measurements of 16-week-old inoculated and uninoculated black oak seedlings receiving starch and IBA treatments

Treatment	No. of primary laterals	No. of mycorrhizal primary laterals	% lateral infection	Infection points per lateral	Dry weight (g)		
					Total root	Taproot	Lateral root
Inoculated							
Control	102*c*	75*a*	73.5*a*	38*a*	4.07*a*	3.16*a*	0.91*b*
Starch	105*c*	82*a*	78.0*a*	35*a*	4.04*ab*	2.87*a*	1.17*a*
Starch–IBA	256*b*	80*a*	31.0*b*	5*b*	2.68*cd*	1.75*b*	0.93*b*
Uninoculated							
Control	116*c*	0*b*	0*c*	0*c*	4.00*ab*	3.19*a*	0.81*b*
Starch	117*c*	0*b*	0*c*	0*c*	3.30*b*	2.83*a*	0.87*b*
Starch–IBA	302*a*	0*b*	0*c*	0*c*	3.22*c*	2.06*b*	1.16*a*

NOTE: Means followed by the same letter are not significantly different at $\alpha = 0.05$.

IBA application to black walnut roots. Seemingly, IBA treatment results in the development of a root system that is more efficient in supporting the growth of the seedling. No comparable published data are available which can be used to compare the effects of IBA on carbon partitioning in mycorrhizal seedlings. Further study of the growth of IBA-treated, container-grown oak seedlings using growth analysis techniques would provide an improved understanding of the role of IBA and ectomycorrhizae in carbon partitioning within the plant. Studies of this nature could provide a more fundamental understanding of the feedback mechanisms involved in the growth of oak seedlings and provide insight into means of improving the growth and development of oak upon outplanting.

AZCON, R., C. AZCON, G. DE AQILAR, and J. M. BAREA. 1978. Effects of plant hormones present in bacterial cultures on the formation and responses to VA endomycorrhizae. New Phytol. **80**: 359–364.

BONNER, J., and A. W. GALSTON. 1952. Principles of plant physiology. W. H. Freeman and Co., San Francisco. p. 55.

CARLSON, W. C. 1974. Root initiation induced by root pruning in northern red oak. Ohio Agric. Res. Dev. Cent. Res. Sum. **74**: 14–16.

CROWNOVER, R. A. 1983. The effects of plant growth regulators on root development and *Pisolithus tinctorius* mycorrhizal colonization of container-grown black oak seedlings. M.S. thesis, University of Missouri, Columbia.

DAUGHTRIDGE, A. T., S. R. BOESE, S. G. PALLARDY, and H. E. GARRETT. 1986. A rapid staining technique for assessment of ectomycorrhizal infection of oak roots. Can. J. Bot. **64**: 1101–1103.

DIXON, R. K., H. E. GARRETT, G. S. COX, P. S. JOHNSON, and I. L. SANDER. 1981. Container and nursery grown black oak seedlings inoculated with *Pisolithus tinctorius*: growth and ectomycorrhizal development following outplanting on an Ozark clear-cut. Can. J. For. Res. **11**: 492–496.

DIXON, R. K., G. M. WRIGHT, G. T. BEHRENS, R. O. TESKEY, and T. M. HINCKLEY. 1980. Water deficits and root growth of ectomycorrhizal white oak seedlings. Can. J. For. Res. **10**: 545–548.

FOWELLS, H. A. 1943. The effect of certain growth substances on root-pruned ponderosa pine seedlings. J. For. **41**: 685–686.

GARRETT, H. E., G. S. COX, R. K. DIXON, and G. M. WRIGHT. 1979. Mycorrhizae and the artificial regeneration potential of oak. *In* Regenerating oaks in upland hardwood forests. Proceedings of the 1979 J. S. Wright Forest Conference, Purdue University, West Lafayette, IN, February 22 and 23, 1979. *Edited by* H. A. Holt and B. C. Fischer. Purdue University, Lafayette. pp. 82–90.

HARLEY, J. L., and S. E. SMITH. 1983. Mycorrhizal symbiosis. Academic Press, London. p. 308.

HARTWIG, R. C., and M. M. LARSON. 1980. Hormone root-soak can increase initial growth of planted hardwood stock. U.S. For. Serv. Tree Plant. Notes, **31**: 29–33.

JOHNSON, P. S. 1974. Containerization of oak seedlings for the oak–hickory region—a progress report. *In* Proceedings of the North American Containerized Forest Tree Seedling Symposium, August 26–29, 1974. *Edited by* R. W. Tinus, W. I. Stein, and W. E. Balmer. Great Plains Agric. Counc. Publ. No. 68. pp. 104–111.

——— 1984. Responses of planted northern red oak to three overstory treatments. Can. J. For. Res. **14**: 536–542.

KORMANIK, P. 1976. Survival and early growth of containerized and bare root seedlings of cherrybark oak. U.S. For. Serv. Tree Plant. Notes, **27**: 9–10.

MAKI, T. E., and H. MARSHALL. 1945. Effects of soaking with indole-butyric acid on root development and survival of tree seedlings. Bot. Gaz. (Chicago), **107**: 268–276.

MARX, D. H., and W. C. BRYAN. 1975. Growth and ectomycorrhizal development of loblolly pine seedlings in fumigated soil infested with the fungal symbiont *Pisolithus tinctorius*. For. Sci. **21**: 245–254.

MCDOUGALL, J., and J. R. HILLMAN. 1978. Analysis of indole-3-acetic acid using GC–MS techniques. *In* Isolation of plant growth substances. *Edited by* J. R. Hillman. Cambridge University Press, New York. pp. 1–27.

MITCHELL, R. J., G. S. COX, R. K. DIXON, H. E. GARRETT, and I. L. SANDER. 1984. Inoculation of three *Quercus* species with eleven isolates of ectomycorrhizal fungi. II. Foliar nutrient content and isolate effectiveness. For. Sci. **30**: 563–572.

MOSER, B. C. 1978. Progress report–research on root regeneration. New Horizons from the Horticultural Research Institute. pp. 18–24.

PATRIC, J. W., and P. F. WAREING. 1972. Experiments on the mechanism of hormone-directed transport. Plant Growth Subst., Proc. Int. Conf., 7th, 1970. pp. 695–700.

SANDER, I. L., P. S. JOHNSON, and R. F. WATT. 1976. A guide for evaluating the adequacy of oak advance reproduction. U.S. For. Ser. Gen. Tech. Rep. NC-23.

SELBY, C., and D. A. SEABY. 1982. The effect of auxins on *Pinus contorta* seedling root development. Forestry, **55**: 125–135.

VANSAMBEEK, J. W., and W. J. REITVELD. 1983. Preplant indolebutyric acid treatment of outplanted black walnut seedlings. Proc. North Am. For. Biol. Workshop, 7th, 1982. pp. 271–280.